

The University of Chicago

CALCIUM AND PHOSPHORUS
METABOLISM OF NORMAL
PRE-SCHOOL CHILDREN

A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE BIO-
LOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF HOME ECONOMICS AND HOUSEHOLD ADMINISTRATION

~~1934~~

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By
THELMA PORTER-LEVIN

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CALCIUM AND PHOSPHORUS METABOLISM OF NORMAL PRE-SCHOOL CHILDREN: I. ON DIETS CONTAINING PLAIN AND IRRADIATED CEREALS¹

THELMA PORTER-LEVIN

AT THE time when interest in the use of irradiated cereals as a source of vitamin D was first aroused, two experiments were carried out to study the effect of these foods on the metabolism of calcium and phosphorus by normal pre-school children. These investigations were made in Chicago during the winter months (December to March) when the ultraviolet rays in the sunlight of the city are low. The subjects were two healthy, well-developed children, a boy four, and a girl two and one-half years old.

In the first experiment short tests were conducted according to the following calendar:

- a. A 5-day preliminary period on *non-irradiated* cereal diet
- b. A 5-day test period on *non-irradiated* cereal diet
- c. A 5-day test period on *irradiated* cereal diet
- d. A 5-day test period on *irradiated* cereal diet

This schedule of feeding was repeated three times.

The basal diet, kept the same each day, included those foods to which the children were accustomed, served in weighed portions; but the food was taken *ad libitum* except for milk (450 gms. per day) and cereals (approximately 10 per cent of the dry weight of the diet per day). This diet furnished an average of 1250 calories per day and was adequate in all respects except that it was low in calcium and vitamin D. These two constituents were restricted in order that any calcifying effect of the activated cereals might be easily detected.

During the experimental feeding the children were in their own home with the usual individuals in charge so that there was no disturbance in environment or discipline. Nine calcium and phosphorus balances were determined on each child. The methods of collections and analyses were essentially the same as described in other studies (1 and 2) of this type. Aliquot samples of the weighed food-intake, and all of the urine and feces excreted, were made into five-day composites for analysis. Food and feces were dried to a constant weight at 80°C. and ground

¹ From the Nutrition Laboratory of the Department of Home Economics of The University of Chicago. The expense of this research was defrayed by a grant from the Quaker Oats Company, Chicago.

TABLE 1

Average calcium and phosphorus storage of four-year-old boy (weight 16.5 kg.), in grams per kilo per day

EXPERIMENT I	OATMEAL		CALCIUM			PHOSPHORUS		
	Kind	Per cent dry diet	Intake	Output	Retention	Intake	Output	Retention
A 1*	Plain	(preliminary period)						
2	Plain	10	0.043	0.031	0.012	0.051	0.039	0.012
3	Irradiated	12	0.045	0.032	0.013	0.051	0.044	0.007
4	Irradiated	10	0.046	0.034	0.012	0.053	0.041	0.012
B 1	Plain	(preliminary period)						
2	Plain	11	0.047	0.033	0.014	0.048	0.040	0.008
3	Irradiated	13	0.033	0.032	0.001	0.045	0.043	0.002
4	Irradiated	11	0.043	0.029	0.014	0.047	0.039	0.008
C 1	Plain	(preliminary period)						
2	Plain	14	0.034	0.027	0.007	0.047	0.027	0.020
3	Irradiated	14	0.040	0.030	0.010	0.048	0.021	0.027
4	Irradiated	12	0.036	0.025	0.011	0.049	0.028	0.021

* All periods were five days long.

TABLE 2

Average calcium and phosphorus storage of two-year-old girl (weight 15.0 kg.), in grams per kilo per day

EXPERIMENT I	OATMEAL		CALCIUM			PHOSPHORUS		
	Kind	Per cent dry diet	Intake	Output	Retention	Intake	Output	Retention
A 1*	Plain	(preliminary period)						
2	Plain	10	0.043	0.036	0.007	0.047	0.038	0.009
3	Irradiated	11	0.041	0.033	0.008	0.050	0.037	0.013
4	Irradiated	10	0.045	0.035	0.010	0.049	0.040	0.009
B 1	Plain	(preliminary period)						
2	Plain	8	0.039	0.031	0.008	0.045	0.036	0.009
3	Irradiated	9	0.038	0.029	0.009	0.043	0.034	0.009
4	Irradiated	9	0.040	0.036	0.004	0.041	0.029	0.012
C 1	Plain	(preliminary period)						
2	Plain	11	0.040	0.029	0.011	0.048	0.030	0.018
3	Irradiated	12	0.043	0.039	0.004	0.052	0.034	0.018
4	Irradiated	11	0.043	0.029	0.014	0.050	0.027	0.023

* All periods were five days long.

finely before being ashed. The calcium was determined in the urine directly, and in the food and feces when dry-ashed at 450°–500°C. by the McCrudden method (4) with the control of the hydrogen-ion concen-

tration for the precipitation of calcium oxalate suggested by Shohl (6). The phosphorus determinations were made according to the double precipitation method of McCandless and Burton (3) on samples which had been digested with sulphuric and nitric acids. All figures in a mineral balance are the average of three analyses which agreed within 2 per cent.

Tables 1 and 2 show the kind and amount of cereal incorporated in the basal diet during each period, and the calcium and phosphorus balances for each subject.

An examination of the balances shows that the mineral intakes varied slightly from period to period, but the retentions fluctuated markedly with no consistent relation to intake or to the introduction of irradiated food containing vitamin D. The retentions of calcium and phosphorus per kilogram of body weight were found to be near the "average" level for subjects of these ages, according to the findings of Sherman and Hawley (5) on normal children, but showed no true differences in favor of the irradiated cereal as a source of vitamin D.

SECOND EXPERIMENT

Because of the conviction that longer dietary periods would have given the body a better opportunity for adjustment to changes in intake and therefore would have given more clear-cut results, a second series of experiments was conducted the following winter on the same subjects. A plan of feeding was carried out as follows:

Period I. Non-irradiated cereals (48 days)

	<i>days</i>
a. Basal diet.....	15
Test.....	5
b. Basal diet.....	23
Test.....	5
	—
Total.....	48

Period II. Irradiated cereals (39 days)

a. Basal diet.....	14
Test.....	5
b. Basal diet.....	15
Test.....	5
	—
Total.....	39

The total of both periods was 87 days.

The purpose of the foregoing experimental calendar was to allow the ingestion of a basal diet containing one or the other type of cereal over a

much longer time than is usually considered necessary. The diet was taken *ad libitum* during the preliminary and intermediate periods, except for milk, butter, and cereal which were kept constant. In the collection periods all food items were served in weighed portions and were kept constant from day to day in a given period.

A child's ordinary mixed diet containing either plain or irradiated cereals was used during the entire eighty-seven days of experimental feeding. It was adequate in all the essential nutrients except that it was rather low in calcium and vitamin D. The average daily caloric intake was between 1250 and 1300, with protein furnishing 11 per cent, fat 31 per cent, and carbohydrate 57 per cent of this energy. The cereals² fed each day amounted to an average of 20 per cent of the calories or 26 per cent of the dry weight of the diet. Four calcium and phosphorus balances were determined on each child and are given in tables 3 and 4. The methods of collection and analyses were the same as used in the first study.

A glance at tables 3 and 4 shows higher retentions of calcium and phosphorus for both subjects during the periods when irradiated cereals were incorporated in the diet. If the average mineral retentions on the two diets are compared, the differences appear at first to be significantly in favor of the periods when activated cereals were used. The average retentions per kilo of body weight were 0.004 gm. and 0.007 gm. per day on plain cereals, and 0.013 gm. and 0.010 gm. per day on irradiated cereals, for calcium and phosphorus respectively. An inspection of the individual balances, however, shows that the variations from period to period, regardless of the cereal used, are as great as the average increases observed on treated cereals. Furthermore the high retentions (0.018 and 0.014 gm. Ca; 0.013 and 0.012 gm. P) obtained in the first balances

² The vitamin D potency of the irradiated cereals used in the diet was evaluated by Dr. F. L. Gunderson of the Quaker Oats Company Nutrition Laboratory by means of thirty-five day feeding tests on groups of standard rats. These determinations indicate that the irradiated cereals possessed some calcifying properties.

Increases in per cent ash of rat femurs corresponding to increases in per cent irradiated cereal as it replaces non-irradiated cereal in rat ration

KIND OF CEREAL	PER CENT ASH IN FEMURS OF 4 RATS AFTER 35 DAYS ON TEST DIET			
	0.0 per cent irradiated cereal	9.5 per cent irradiated cereal	19.0 per cent irradiated cereal	76 per cent irradiated cereal
Oatmeal.....	36.9	43.6	49.9	54.8
Farina.....	41.7	43.8	50.5	50.5

TABLE 3

Average calcium storage of children during the last five days of a twenty-day period on a diet containing plain or irradiated cereals, in grams per kilo per day

SUBJECT			CEREAL			CALCIUM			
Sex	Age		Weight	Kind	Per cent dry diet	Intake per kilogram	Output per kilogram	Retention	
	Years	Months						Per kilogram	Per cent of intake
			kg.			gm.	gm.	gm.	
Boy	5	2	18.5	Non-irradiated	24	0.039	0.035	0.004	10
Boy	5	3	18.4	Non-irradiated	31	0.039	0.037	0.002	5
Girl	3	6	17.7	Non-irradiated	23	0.042	0.039	0.003	7
Girl	3	7	17.3	Non-irradiated	32	0.041	0.036	0.005	12
Average.....			18.0	Non-irradiated	28	0.040	0.036	0.004	9
Boy	5	4	18.9	Irradiated	27	0.046	0.028	0.018	39
Boy	5	5	19.1	Irradiated	25	0.037	0.028	0.009	24
Girl	3	8	17.7	Irradiated	29	0.045	0.031	0.014	31
Girl	3	9	17.9	Irradiated	24	0.042	0.033	0.009	21
Average.....			18.4	Irradiated	26	0.042	0.027	0.013	29

TABLE 4

Average phosphorus storage of children during the last five days of a twenty-day period on diets containing plain or irradiated cereals, in grams per kilo per day

SUBJECT			CEREAL			CALCIUM			
Sex	Age		Weight	Kind	Per cent dry diet	Intake per kilogram	Output per kilogram	Retention	
	Year	Months						Per kilogram	Per cent of intake
			kg.			gm.	gm.	gm.	
Boy	5	2	18.5	Non-irradiated	24	0.052	0.044	0.008	15
Boy	5	3	18.4	Non-irradiated	31	0.048	0.045	0.003	6
Girl	3	6	17.7	Non-irradiated	23	0.055	0.045	0.010	18
Girl	3	7	17.3	Non-irradiated	32	0.048	0.042	0.006	12
Average.....			18.0	Non-irradiated	28	0.051	0.044	0.007	13
Boy	5	4	18.9	Irradiated	27	0.049	0.036	0.013	27
Boy	5	5	19.1	Irradiated	25	0.044	0.039	0.005	11
Girl	3	8	17.7	Irradiated	29	0.052	0.040	0.012	23
Girl	3	9	17.9	Irradiated	24	0.048	0.037	0.011	23
Average.....			18.4	Irradiated	26	0.048	0.038	0.010	21

following the introduction of irradiated cereals are not maintained until the next test period fifteen days later. These last balances were considerably lower, although the irradiated cereals were still being ingested.

These observations at once raise the questions as to what changes in the level of storage there may have been between these 'sampling' periods, and what factors caused the variations from period to period. While the first impulse is to conclude that the mineral storage was improved during the feeding of the treated cereals, more careful study of the findings, with due consideration of their fluctuations, leads to the realization that this may not be justified. Although the change in the type of cereal in the diet was the only variable known, the fluctuations in the results may have been accentuated by unknown and uncontrollable influences on the mineral metabolism of these human subjects, or may merely indicate the 'normal' variations in mineral storage. Further, since the balances were not continuous in their measurement of the changes occurring in the bodies of the children, it is impossible to determine the extreme ranges of variation in mineral storage which may have occurred.

Therefore in spite of the fact that in this second experiment some increases in mineral retentions were observed during the feeding of the irradiated cereals, and that these changes are of the same order of magnitude as differences commonly reported as significant, it was felt that the interpretation of the results should be withheld until the mineral metabolism of children had been studied continuously over a considerable period of time; some understanding of the extent of 'normal' variation would then be available. It was the need for data on this question that led to the experiment to be reported later. As will be seen in the light of those results, the significance of the findings on the two types of cereal diets can not be determined until the effects of such diets have been tested during longer, successive balance studies.

SUMMARY

Two normal pre-school children were fed controlled mixed diets, furnishing approximately 50 mg. each of calcium and phosphorus per kilo per day, and containing either plain or irradiated cereals. Twenty-six calcium and phosphorus balances were determined. In the first study employing short dietary periods, the mineral retentions showed no improvement in favor of the irradiated cereal diet. In the second study, when longer periods on a given diet were sampled by five-day balances, the average retentions per kilo per day were 0.004 gm. and 0.007 gm.

on plain cereals, and 0.013 gm. and 0.010 gm. on irradiated cereals for calcium and phosphorus respectively.

While the differences in average mineral retentions in this last experiment appear to show increases in favor of the balances when irradiated cereals were fed, the variations of 1 to 9 mg. from period to period, regardless of the diet, are as great as the average increases of 3 to 8 mg. observed on treated cereals. The explanation of the prevalence of retention variations throughout the study is not clearly understood, especially when the only known variable in the exacting management of the subjects was the change in the cereal of the basal diet. Therefore it appears that conclusions regarding the significance of these findings should be withheld until the effects of two types of cereal on mineral storage can be determined over longer periods by means of continuous balances. The difficulty of interpreting the results points to the need for the study of successive mineral balances in children in order that the extent of the 'normal' or expected variations in mineral storage may be understood. Such a study will be reported in a following paper.

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IES SHOWING THE RANGE OF VARIATION IN CALCIUM
AND PHOSPHORUS STORAGE

CALCIUM AND PHOSPHORUS METABOLISM OF NORMAL PRE-SCHOOL CHILDREN: II. SUCCESSIVE BALANCE STUDIES SHOWING THE RANGE OF VARIATION IN CALCIUM AND PHOSPHORUS STORAGE

THELMA PORTER-LEVIN

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THE investigation reported in this paper is a study of successive mineral balances determined on normal pre-school children living in their usual home environment and eating their usual diet. The incentive for this analysis was secured primarily from two previous experiments (8) in which an attempt was made to compare the effects of plain and irradiated cereals on the mineral storage levels of children from two to five years old. The results from this earlier work showed marked variations in the quantity of calcium and phosphorus retained per kilogram of body weight in subjects of this age, even when the values were secured from five-day samplings taken in the middle and at the end of forty days on a relatively constant diet. From the experimental procedures used, it seemed that the explanation of such variations, amounting to as much as 25 milligrams per kilo per day in some cases, could be found only in the normal metabolic processes.

An examination of the literature revealed that studies on the mineral metabolism of normal children of pre-school age are not numerous. In those studies in which the discussions indicate that the subjects were normal and the experimental conditions well-controlled (1, 2, 3, 5, 10, 11), a consideration of the quantitative data showed that large variations in the actual amounts of calcium and phosphorus retained were frequently recorded. Usually from 1 to 3 days were used as preliminary periods, during which time it has been assumed that the body adjusted itself to a "steady level" of metabolism on a new dietary regimen. No *continuous* study of the normal mineral metabolism over a considerable period of time without the introduction or removal of some test substance seemed to be available.

The desire to discover the extent and nature of the occurrence of variations in the mineral metabolism of normal children, as well as to determine the period of time for study which would include such fluctuations, prompted this study. It was felt that continuous balances of calcium and phosphorus, taken under normal but well-controlled conditions over a period of 60 days, would give a series of retention rates

which would be a contribution toward the determination of "normal" levels of mineral storage for children from 2 to 6 years old.

EXPERIMENTAL PROCEDURE

The subjects were healthy, in good nutritional condition, and included a boy, O, 2 years and 7 months old, weighing 15 kilograms; a girl, M, 4 years and 8 months old, weighing 18 kilograms; and another girl, J, 5½ years old, weighing 23 kilograms. The children were cared for in home surroundings under the supervision of their mothers. Regular visits to the nursery school physician, with whom the children were acquainted, constituted a pleasant and adequate medical supervision. It is important to note that throughout the course of the experiment the subjects evidenced no unusual disturbance of an emotional or disciplinary nature, and lived with but very little change in environment due to the balance study procedures. Every attempt was made to maintain conditions to which the children were accustomed.

The experimental calendar was made up of a series of successive three-day balance periods, beginning February 2, 1932, and continuing through April 1 of the same year. This totaled 60 days and included 20 three-day periods. The older girl, J, served as a subject for the 20 periods; the boy, O, was studied for 17 of the 20 periods, 15 of which were consecutive. The third child, M, was available for only 6 periods.

The effort to observe the usual and "normal" condition was carried into the planning of the diet, which included only those foods which the children liked. Meals were planned for 3 successive days, and tried out for 2 three-day periods; the intakes were adjusted to the usual appetite and requirement of each subject as nearly as possible. After such quantities were determined, the same three-day meal schedule was repeated successively throughout the study, and, as one of the significant points in the experimental procedure, the intakes were kept constant. The exact daily food and water intakes of each subject are recorded in table 1.

For analytical work, aliquot samples of the weighed food intakes and of all of the excreted urine and feces were made into three-day composites. Food and feces were dried to a constant weight at 80°C. and finely ground before being ashed. The incineration of all materials was accomplished satisfactorily by an overnight heating of weighed portions in an electric furnace at 450°C. Calcium was precipitated as calcium oxalate by the McCrudden method (7), and the quantity determined by titration with N50 potassium permanganate. Phosphorus was determined by the colorimetric method of Fiske and Subbarow (6). All figures

TABLE 1
Daily food intakes of three children during a three-day dietary unit in a sixty-day balance study

FIRST DAY				SECOND DAY				THIRD DAY			
Food				Food				Food			
Grams per day				Grams per day				Grams per day			
O	M	J		O	M	J		O	M	J	
Milk, whole.....	720	720	720	Milk, whole.....	695	695	695	Milk, whole.....	720	720	720
Bread.....	30	57	68	Bread.....	30	57	68	Bread.....	30	57	68
Butter.....	23	23	23	Butter.....	24	24	24	Butter.....	24	24	24
Orange juice.....	100	100	100	Orange juice.....	100	100	100	Orange juice.....	100	100	100
Oatmeal porridge.....	100	100	100	Rice "Pops".....	10	10	15	Cream of wheat.....	100	100	100
Graham crackers.....	16--	16--	24--	Graham crackers.....	16--	16--	24--	Graham crackers.....	16--	16--	24--
Tomato-spaghetti.....	24	24	32	Potato, baked.....	24	24	32	Beef stew.....	24	24	32
Canned apricot {fruit	80	80	170	Canned pears {fruit	40	40	40	Canned peaches {fruit	75	75	150
Apple sauce.....	15	50	45	Spinach.....	60	100	100	Jello.....	60	60	90
Bacon, cooked.....	100	100	150	Beef, lean.....	30	50	50	Bacon, cooked.....	30	30	45
Carrots, cooked.....	10	10	15	Bacon, cooked.....	40	20	40	Green beans.....	50	70	100
Sugar.....	35	35	50	Peas, creamed.....	40	40	60	Sugar.....	10	10	15
Vitavose.....	8	8	8	Bananas, E. P.....	10	10	15	Water, distilled.....	35	50	50
Water, distilled.....	4	4	4	Sugar.....	60	60	75		8	8	8
	500	750	300	Water, distilled.....	60	60	90		500	750	300
					8	8	8				
					500	750	300				
Calories.....	1,404	1,537	1,680		1,349	1,462	1,653		1,321	1,416	1,620

in a balance represent averages of three analyses which agreed within 2 per cent.

The mineral intakes, excretions, and storages were tabulated for statistical treatment. While it was fully recognized that a general and uncritical application of reliability formulas leads to erroneous conclusions, it was felt that, because of the degree of control exercised in the compilation of the data, some measure of the true "representativeness" of the sample was of interest and perhaps was justifiable. The standard deviation of each series was determined, as well as the mean with its probable error, and the coefficient of variation. A frequency distribution of averages of consecutive balances within the range of $\pm 4PE$ of the mean was determined on the two longer series of retentions in order to make an estimate of the number of three-day balances which would include the observed variations.

The nitrogen balances, which showed that all the children were storing nitrogen throughout the study, were determined by the Kjeldahl method and will be discussed in a later paper.

DISCUSSION

Mineral intakes. The mineral intakes are of especial interest in this study, since the level of intake with its variations has a significant and direct relation to the storage of these minerals. In order to be able to study variations in retentions it was necessary to keep under exact control the conditions of feeding and housing the subjects, and particularly to maintain in a given subject a fixed level of calcium and phosphorus ingestion. The carefully weighed and prepared diet, as well as the good health and coöperation of the subjects, made this possible.

Intakes which were maintained at remarkably constant levels are illustrated by those of subject J. The total calcium intake of this 23 kilogram girl averaged 1.010 ± 0.004 gms. per day, with a range of 0.090 gm. The entire series of twenty intakes had a standard deviation of 26 mgs. and a coefficient of variation of 2.6. Similarly, the total intakes of phosphorus for J averaged 1.050 ± 0.004 gms. per day, with a range of 0.090 gm. The standard deviation was 24 mgs. in this series of intakes, and the coefficient of variation 2. The intakes of calcium and phosphorus for the other two children were nearly the same as those for J (see tables 2 and 3), and all were sufficiently constant to warrant a subsequent analysis of variations in retentions.

Mineral excretions. The usual paths of excretion of the minerals calcium and phosphorus are exemplified in the data secured from these

TABLE 2

Average calcium balances in grams per day from successive three-day periods in a sixty-day study on three children

PERIOD	INTAKE		EXCRETION				RETENTION	
	Total	Per kilo	Urine	Feces	Total	Per kilo	Total	Per kilo

Girl J, 5 yrs. 6 mos. old, weighing 22.5-24.2 kg.

	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
1	0.99	0.044	0.06	0.91	0.97	0.043	0.02	0.001
2	1.05	0.046	0.10	0.91	1.01	0.044	0.04	0.002
3	0.98	0.043	0.05	0.83	0.88	0.038	0.10	0.005
4	1.02	0.044	0.04	0.87	0.91	0.039	0.11	0.005
5	1.00	0.043	0.03	0.83	0.86	0.037	0.14	0.006
6	0.98	0.043	0.03	0.85	0.88	0.038	0.10	0.005
7	0.96	0.041	0.03	0.84	0.87	0.037	0.09	0.004
8	0.96	0.040	0.02	0.89	0.91	0.038	0.05	0.002
9	1.03	0.043	0.03	0.86	0.89	0.037	0.14	0.006
10	1.01	0.043	0.02	0.81	0.83	0.035	0.18	0.008
11	1.03	0.043	0.02	0.80	0.82	0.034	0.21	0.009
12	1.02	0.043	0.03	0.87	0.90	0.038	0.12	0.005
13	1.01	0.042	0.03	0.84	0.87	0.036	0.14	0.006
14	1.00	0.042	0.03	0.92	0.95	0.039	0.05	0.003
15	1.02	0.042	0.04	0.86	0.90	0.037	0.12	0.005
16	1.02	0.043	0.03	0.91	0.94	0.039	0.08	0.004
17	1.02	0.043	0.03	0.83	0.86	0.035	0.16	0.008
18	1.05	0.043	0.03	0.94	0.97	0.040	0.08	0.003
19	1.03	0.043	0.03	0.86	0.89	0.036	0.14	0.007
20	1.03	0.042	0.03	0.81	0.84	0.034	0.19	0.008
Mean...	1.01	0.043	0.04	0.86	0.90	0.038	0.11	0.005

Boy O, 2 yrs. 7 mos. old, weighing 14.6-15.8 kg.

1	0.96	0.066	0.07	0.66	0.73	0.050	0.23	0.016
2	0.98	0.067	0.10	0.62	0.72	0.049	0.26	0.018
6	0.93	0.065	0.09	0.73	0.82	0.057	0.11	0.008
7	0.97	0.067	0.09	0.83	0.92	0.064	0.05	0.003
8	0.94	0.064	0.10	0.82	0.92	0.063	0.02	0.001
9	0.97	0.067	0.09	0.79	0.88	0.060	0.09	0.007
10	0.97	0.065	0.09	0.68	0.77	0.052	0.20	0.013
11	0.99	0.067	0.09	0.80	0.89	0.060	0.10	0.007
12	0.98	0.065	0.09	0.76	0.85	0.056	0.14	0.009
13	0.97	0.064	0.09	0.81	0.90	0.059	0.07	0.005
14	0.99	0.065	0.08	0.72	0.80	0.052	0.19	0.013
15	0.99	0.064	0.08	0.82	0.90	0.058	0.09	0.006
16	0.97	0.062	0.07	0.78	0.85	0.055	0.12	0.007
17	0.95	0.062	0.08	0.70	0.78	0.051	0.17	0.011
18	0.98	0.062	0.08	0.66	0.74	0.047	0.24	0.015
19	0.96	0.061	0.08	0.77	0.85	0.054	0.11	0.007
20	0.97	0.061	0.07	0.71	0.78	0.049	0.19	0.012
Mean...	0.97	0.064	0.09	0.74	0.83	0.055	0.14	0.009

TABLE 2—*Concluded*

PERIOD	INTAKE		EXCRETION				RETENTION	
	Total	Per kilo	Urine	Feces	Total	Per kilo	Total	Per kilo
Girl M, 4 yrs. 8 mos. old, weighing 17.7 kg.								
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
1	0.97	0.055	0.10	0.70	0.80	0.045	0.17	0.010
2	1.00	0.056	0.12	0.74	0.86	0.048	0.14	0.008
3	0.94	0.052	0.09	0.72	0.81	0.045	0.13	0.007
4	0.94	0.052	0.11	0.68	0.79	0.044	0.15	0.008
7	0.97	0.055	0.07	0.89	0.96	0.054	0.01	0.001
8	0.96	0.054	0.08	0.73	0.81	0.045	0.15	0.009
Mean...	0.96	0.054	0.10	0.74	0.84	0.047	0.13	0.007

children. Most of the calcium was excreted in the feces, and only a small, relatively constant amount in the urine. On the other hand, a slightly larger portion of the total phosphorus excretion appeared in the urine, with somewhat smaller amounts in the feces. The total excretion of both these minerals, amounting to 87 per cent for calcium and 83 per cent for phosphorus, constituted considerable portions of the intakes. This fact, coupled with the occurrence of variations in excretions more than twice as great as those appearing in the intakes, points to the reason for the fluctuations in the retention levels.

Mineral retentions. It is in the analysis of the findings, on the retention levels of these minerals in the children, that the greatest interest lies. In general it may be stated that the diet in this study allowed for the retention of minerals at a normal level for children of these ages (4, 9). The total calcium retention of all subjects averaged 13 per cent, and the phosphorus retention 17 per cent of the respective intakes of these minerals. On the mixed diet, furnishing approximately one gram each of calcium and phosphorus per day, these two, four, and five-year-old children stored, respectively, 0.14 gm., 0.13 gm., and 0.11 gm. of calcium, and 0.16 gm., 0.13 gm., and 0.14 gm. of phosphorus per day. The average retentions per kilo of body weight, for the subjects in the same order, were 0.009 gm., 0.007 gm., and 0.005 gm. of calcium, and 0.010 gm., 0.007 gm., and 0.006 gm. of phosphorus per day. The average "per kilo" retentions, as represented by the mean of the entire series of 43 balances, were 0.007 gm. of calcium and 0.008 gm. of phosphorus per day. These average retention values, given in tables 2 and 3, show that the levels of storage of both calcium and phosphorus are about the same in all the

TABLE 3

Average phosphorus balances in grams per day from successive three-day periods in a sixty-day study on three children

PERIOD	INTAKE		EXCRETION				RETENTION	
	Total	Per kilo	Urine	Feces	Total	Per kilo	Total	Per kilo

Girl J, 5 yrs. 6 mos. old, weighing 22.5-24.2 kg.

	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
1	1.01	0.044	0.49	0.49	0.98	0.043	0.03	0.001
2	1.07	0.047	0.49	0.44	0.93	0.041	0.14	0.006
3	1.02	0.045	0.49	0.36	0.85	0.037	0.17	0.008
4	1.06	0.046	0.49	0.37	0.86	0.037	0.20	0.009
5	1.06	0.046	0.46	0.39	0.85	0.037	0.21	0.009
6	1.06	0.046	0.55	0.40	0.95	0.041	0.11	0.005
7	1.03	0.045	0.47	0.45	0.92	0.040	0.11	0.005
8	1.07	0.045	0.52	0.40	0.92	0.039	0.15	0.006
9	1.06	0.045	0.49	0.36	0.85	0.036	0.21	0.009
10	1.00	0.043	0.51	0.35	0.86	0.037	0.14	0.006
11	1.03	0.044	0.56	0.34	0.90	0.038	0.13	0.006
12	1.06	0.045	0.56	0.36	0.92	0.039	0.14	0.006
13	1.01	0.043	0.54	0.34	0.88	0.037	0.13	0.006
14	1.04	0.043	0.58	0.33	0.91	0.038	0.13	0.005
15	1.05	0.044	0.56	0.34	0.90	0.038	0.15	0.006
16	1.06	0.044	0.53	0.40	0.93	0.039	0.13	0.005
17	1.05	0.044	0.57	0.35	0.92	0.039	0.13	0.005
18	1.04	0.043	0.58	0.39	0.97	0.040	0.07	0.003
19	1.05	0.043	0.56	0.34	0.90	0.037	0.15	0.006
20	1.09	0.045	0.53	0.37	0.90	0.037	0.19	0.008
Mean...	1.05	0.045	0.53	0.38	0.91	0.039	0.14	0.006

Boy O, 2 yrs. 7 mos. old, weighing 14.6-15.8 kg.

	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
1	0.89	0.061	0.43	0.32	0.75	0.051	0.15	0.010
2	0.91	0.061	0.45	0.26	0.71	0.048	0.20	0.013
6	0.91	0.063	0.45	0.32	0.77	0.054	0.13	0.009
7	0.91	0.062	0.41	0.40	0.81	0.056	0.10	0.006
8	0.94	0.064	0.52	0.31	0.83	0.056	0.11	0.008
9	0.91	0.062	0.45	0.31	0.76	0.052	0.15	0.010
10	0.88	0.060	0.43	0.27	0.70	0.048	0.18	0.012
11	0.91	0.061	0.46	0.31	0.77	0.052	0.14	0.009
12	0.93	0.061	0.42	0.30	0.72	0.048	0.21	0.014
13	0.90	0.059	0.46	0.32	0.78	0.051	0.12	0.008
14	0.90	0.059	0.50	0.28	0.78	0.051	0.12	0.008
15	0.93	0.060	0.42	0.31	0.73	0.047	0.20	0.013
16	0.90	0.058	0.41	0.31	0.72	0.046	0.18	0.012
17	0.91	0.058	0.46	0.27	0.73	0.046	0.18	0.012
18	0.90	0.057	0.45	0.26	0.71	0.045	0.18	0.012
19	0.90	0.057	0.46	0.28	0.74	0.047	0.17	0.010
20	0.92	0.058	0.46	0.31	0.77	0.048	0.15	0.010
Mean...	0.91	0.061	0.45	0.30	0.75	0.050	0.16	0.011

TABLE 3—*Concluded*

PERIOD	INTAKE		EXCRETION				RETENTION	
	Total	Per kilo	Urine	Feces	Total	Per kilo	Total	Per kilo
Girl M, 4 yrs. 8 mos. old, weighing 17.7 kg.								
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
1	0.92	0.052	0.54	0.31	0.85	0.048	0.07	0.004
2	0.93	0.052	0.53	0.31	0.84	0.047	0.09	0.005
3	0.93	0.052	0.48	0.29	0.77	0.043	0.16	0.009
4	0.95	0.053	0.53	0.26	0.79	0.044	0.16	0.009
7	0.94	0.053	0.42	0.37	0.79	0.045	0.15	0.008
8	0.93	0.052	0.51	0.28	0.79	0.044	0.14	0.008
Mean...	0.93	0.052	0.50	0.30	0.80	0.045	0.13	0.007

subjects, although whenever there was a difference the phosphorus retention averaged slightly higher than the calcium (2 to 4 per cent).

Perhaps the most pertinent observation on the retentions is their variability in a given subject under controlled conditions. While these variations may be emphasized by the low mean level of retention, they appear to be much greater than would be expected from the controlled experimental regimen. For subjects O, M, and J, respectively, the actual ranges of variation in the total daily storage of calcium were 240 mgs., 160 mgs., and 190 mgs.; in total phosphorus storage the variations were 110 mgs., 90 mgs., and 180 mgs. These fluctuations, per kilo of weight for O, M, and J, respectively, were 17 mgs., 9 mgs., 8 mgs. of calcium, and 8 mgs., 5 mgs., and 8 mgs. of phosphorus. In each instance the phosphorus retention was slightly less variable than the calcium.

As evidence that similar variations in mineral retentions, even when the intake is constant, appear as a normal condition, some analyses of figures in the literature may be permissible. General comparisons, of course, are difficult to make because exactly similar experimental conditions are not found. Nevertheless an examination of the quantitative data of two studies is made. Perhaps the best comparisons with this study can be found in the work of Sherman and Hawley (9). In their report (1922) we find two series of successive balances, each with 5 three-day periods in consecutive order, on given subjects. Interpretations of these data to parallel exactly the figures of the present study show for subject L. M., a twelve-year-old girl, an intake of calcium averaging 1.051 ± 0.011 gms. per day, with a coefficient of variation of 3.5. On such an intake we find an average retention of 0.207 ± 0.026 gm. per day, with a coefficient of

variation of 42 and a range of more than 200 mgs. Similarly, on subject A. M., a six-year-old boy, we observe an intake of calcium with a mean level of 0.883 ± 0.003 gm. per day, with a coefficient of variation of only 2. The average retention of calcium was 0.104 ± 0.008 gm. per day, with a coefficient of variation of 29 and a range of 65 mgs. Even wider variations occurred in the phosphorus retentions of the subjects of Sherman and Hawley, since some of the phosphorus balances were negative.

More recently, Boldt, Brahm and Andresen (1) studied the mineral metabolism of two healthy infants on breast and cow's milk by means of successive three-day balances over a period of approximately 90 days. In their investigation we also find marked variations in the retentions of calcium and phosphorus on relatively constant diets. In fact, in only one subject, for one period of 27 days, were there no negative balances. In this subject we find an intake averaging 0.218 ± 0.003 gm. of calcium per day, with a coefficient of variation of 8; on such an intake, variations in retention result in an average storage of 0.057 ± 0.007 gm. per day, a range of 91 mgs., and a coefficient of variation of 58. These two specific comparisons are probably the most exact ones which can be made from the data in the literature, and they indicate that the fluctuations in retentions are of the same order of magnitude as those in the present study.

With these considerations in mind it is pertinent to return to an analysis of the figures in the previous study (8) of the comparison of the effects of plain and irradiated cereals on calcium and phosphorus storage. In that experiment marked changes in the levels of mineral retention, regardless of the kind of cereal used, seemed to make it necessary to reserve the interpretation of the results until further work had been done. At that time no data were available, it was felt, which could be used as a "standard" or measure of normal variations. The findings in the present study, however, seem to offer a series of retentions which may be used in this way and to substantiate the early view that certain fluctuations would normally be observed in the mineral retentions of children on constant diets. A comparison of the amounts of variation in the levels of mineral storage in the two investigations shows that they are similar in range. For example, with respective intakes of calcium of 0.041 gm., 0.042 gm., and 0.053 gm. per kilo per day, and storages of 0.007 gm., 0.10 gm., and 0.007 gm. per kilo per day, the extreme fluctuations of calcium retention were 12 mgs. on plain cereals; 17 mgs. on irradiated cereals; and 17 mgs. on the adequate mixed diet of this last study. The

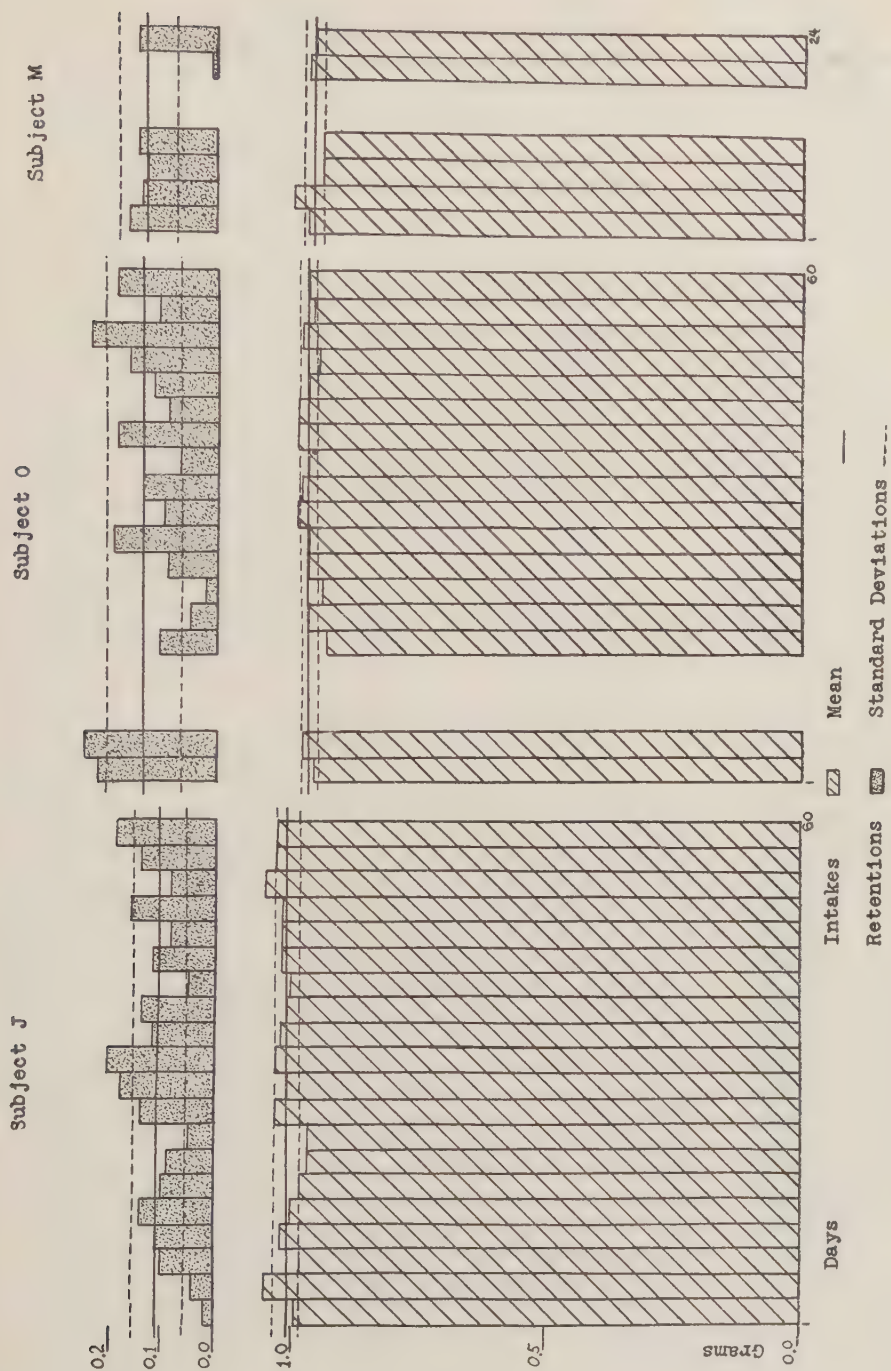


FIG. 1. CALCIUM INTAKES AND RETENTIONS OF THREE CHILDREN

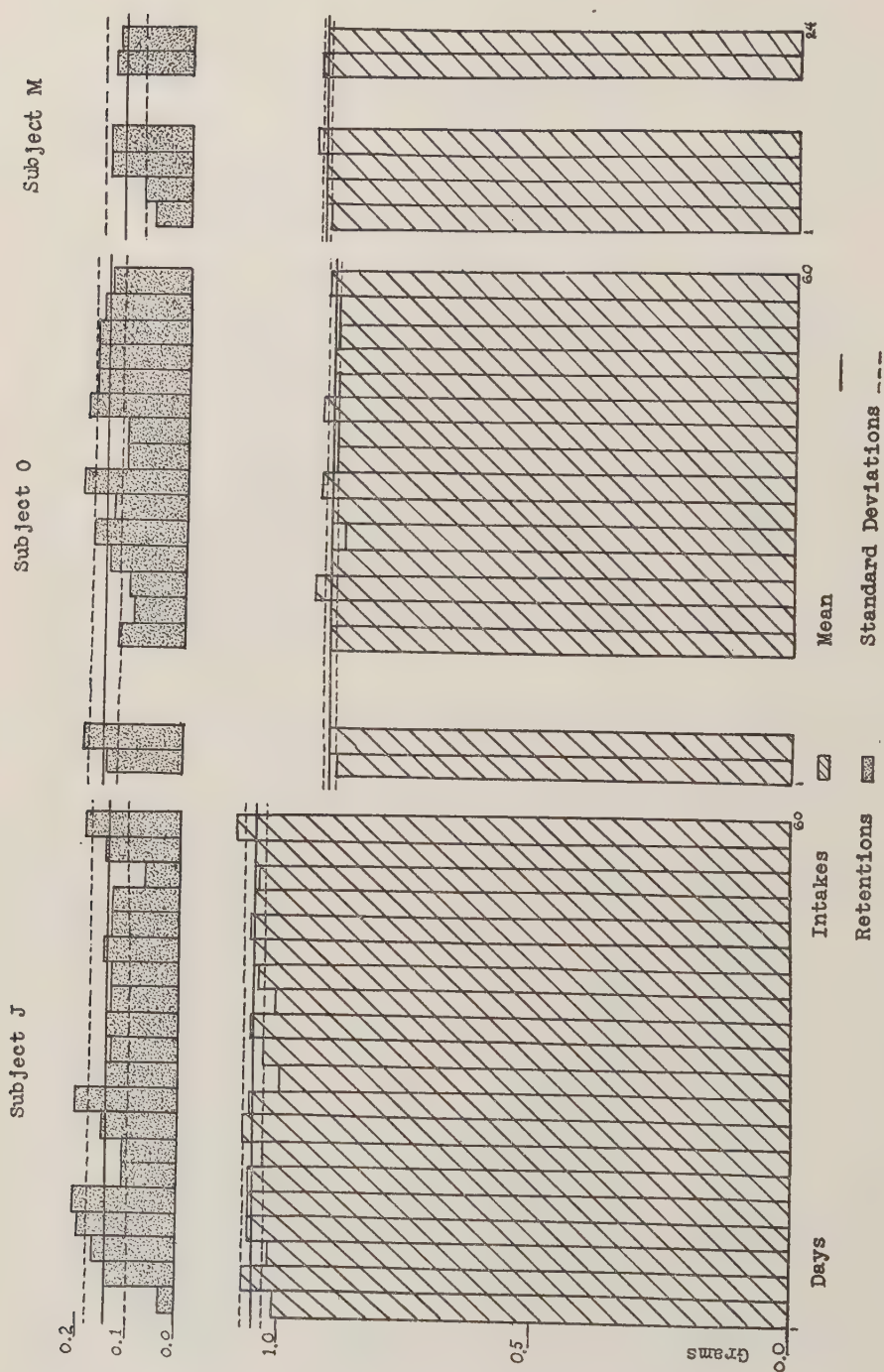


FIG. 2. PHOSPHORUS INTAKES AND RETENTIONS OF THREE CHILDREN

fluctuations in the phosphorus storage paralleled one another in the same manner. The fact that similar ranges of variation in mineral retention appeared in both these studies on pre-school children indicates that they are probably "normal" or expected fluctuations under the conditions of study and technique described.

If wide variations in mineral retentions occur normally in young children on constant diets, this fact is important when considered in the light of its influence on the correct interpretation of mineral storage data. It can be imagined that the selection of random balances as measures of a fixed level of mineral storage in the body may give false values and incorrect interpretations to the effects of a test factor under observation. In reality such chance balances may measure a high or low point within the expected range. Particularly might this be true when there is not due consideration of the changes in the quantities of calcium and phosphorus ingested, or of the starved or saturated condition of body at the time of the test, and when there is not adequate control of the numerous qualitative factors affecting mineral mobilization in the body.

The foregoing consideration of retention variations leads to the question of the length of time for a study which would represent an "adequate sample" of the calcium and phosphorus metabolism of these subjects. A glance at Figures 1 and 2 indicates the somewhat wave-like changes in the levels of storage, and shows that it is not until five to seven balances are included that the complete ranges are covered. As another means of attack on this problem of determining the period of time covering the observed fluctuations, a statistical treatment of the two longer series of retentions was carried out. A frequency distribution of averages of *consecutive* balances within the range of $\pm 4PE$ of the mean of each series was made. These calculations revealed that five to seven balances were required before the range of variations was included, the same length of time that a careful inspection of the charts show. It appears, then, that continuous measurement of the mineral metabolism of these children, under this experimental regimen for a period of from fifteen to twenty-one days, was necessary before a complete picture of the changes in the storage of calcium or phosphorus was obtained. This period of time is considerably longer than has commonly been considered necessary for mineral balance studies on children.

SUMMARY

A study of the range of variation in the retention of calcium and phosphorus has been made from successive mineral balances upon three

normal children, two to six years of age, living under controlled home conditions. Forty-three determinations of the complete intake and output of each mineral were made. In every case some calcium and phosphorus were being stored. Twenty consecutive three-day balances were secured on one child; seventeen balances, fifteen of which were consecutive, on another; and six on a third.

1. On a constant mixed diet, furnishing approximately one gram each of calcium and phosphorus per day, the 2, 4, and 5-year-old children stored respectively a daily average of 0.14 gm., 0.13 gm., and 0.11 gm. of calcium, and 0.16 gm., 0.13 gm., and 0.14 gm. of phosphorus. On a "per kilo" basis, these average retentions were, in the same order, 0.009 gm., 0.007 gm., and 0.005 gm. of calcium, and 0.010 gm., 0.007 gm., and 0.006 gm. of phosphorus per day.

2. The retentions of calcium and phosphorus in each series of successive balances secured under controlled conditions showed wide variations from period to period. The range of variation in the total retentions of calcium was 240, 160, and 190 mgs., in the same subjects, respectively, or on a per kilo basis, 17, 9, and 8 mgs. Likewise, the total phosphorus retentions fluctuated over a range of 110, 90, and 180 mgs., or 8, 5, and 8 mgs. per kilo, in the three children.

3. An inspection, as well as statistical treatment of the two longer series of retentions, indicates that it required from five to seven successive balances, or from fifteen to twenty-one consecutive days, to cover the entire range of variation in these retentions.

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